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ANIMALS IN ETHIOPIA

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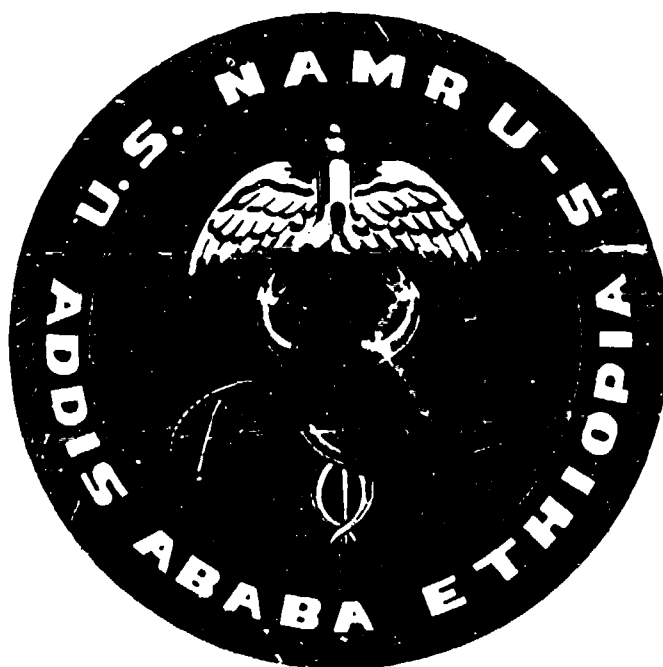
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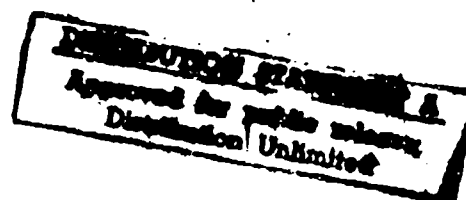
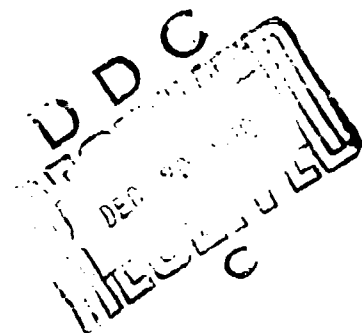
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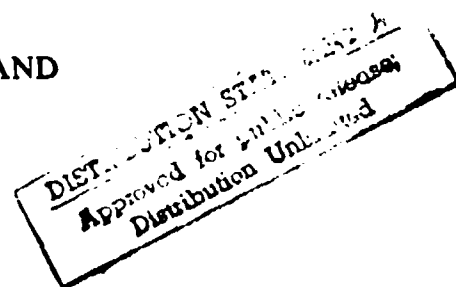
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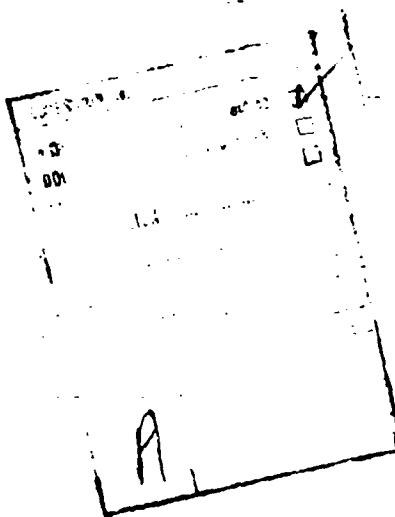
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ADMINISTRATIVE INFORMATION

The opinions and assertions in this report are those of the authors and do not necessarily reflect the official views of the Navy Department or the naval service at large. The observations were made while engaged in work supported by Naval Medical Research and Development Command Work Unit No. MR51.524.009-0018.

The experiments reported herein were conducted according to the principles set forth in "Guide for Laboratory Animal Facilities and Care" prepared by the Committee on the Guide for Laboratory Animal Resources, National Academy of Science, National Research Council.

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LEPTOSPIROSIS SURVEY OF RODENTS AND
DOMESTIC ANIMALS IN ETHIOPIA

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A field study was conducted to survey wild rodents and domestic animals for leptospirosis in 5 sites in western and southern Ethiopia from April 1975 through December 1975. Although leptospirosis is considered to have a world-wide distribution, the incidence of this protean disease, in man and animals in Ethiopia, is almost entirely unknown. This is due, in large part, to the limited medical and veterinary personnel available within the country and to the lack of diagnostic facilities. Because there are significant numbers of fevers, of unknown origin, seen at medical facilities in Ethiopia (Teferra and Abdul Kadir, 1968; Molineux *et al.*, 1966) and because of a close association of natives with rodents, it was felt that the rodents may be a significant reservoir of leptospirosis as they are in other areas of the world (Alexander *et al.*, 1963; Tsai *et al.*, 1971; Sulzer *et al.*, 1968; Ball, 1966). Domestic animals were also sampled in 3 of the areas to determine if these animals may have had exposure to the disease.

MATERIALS AND METHODS

Rodents were trapped live in 5 provinces (Fig. 1). These animals were obtained in and around family dwelling units and from nearby fields. The animals were anesthetized with chloroform and, aseptically, bled by cardiac puncture. The following day the serum was harvested and, to the majority of samples, a 1:1000 solution of merthiolate was added prior to storage. Sera samples were stored at -20°C until serologic testing was performed. Immediately after bleeding, the abdomen was opened with sterile scissors and forceps; a second set of instruments was used to remove, aseptically, a kidney for culture. The kidney was placed in a 2 cc syringe and expelled into a tube of Fletcher's semi-solid media containing either 200 or 300 microgrammes of 5-Fluorouracil per ml of media to inhibit bacterial growth. The following day, 1:100 and 1:1000 dilutions of the culture were made with phosphate buffered saline (pH 7.2) and several drops, of each dilution, were transferred to fresh tubes of media.

Cultures were incubated at 30°C and examined weekly by dark field microscopy for 1 month for evidence of leptospiral growth.

Cattle, sheep, and goats were bled from the jugular vein and the sera handled in the same manner as described for rodents.

The macroscopic slide agglutination test (ST) was performed using commercially prepared formalin killed organisms in a glycerol based media. (DIFCO Laboratories, Detroit, Michigan). The sera were originally screened with 6 pools of antigens containing 3 antigens each. The serotypes represented were as follows:

Pool 1: *ballum*, *canicola*, *icterohemorrhagiae*

Pool 2: *bataviae*, *grippotyphosa*, *pyrogenes*

Pool 3: *autumnalis*, *pomona*, *wolffii*

Pool 4: *australis*, *hyos*, *mini, georgia*

Pool 5: *cynopteri*, *celledoni*, *javanica*

Pool 6: *cynopteri*, *panama*, *shermani*

Any sera showing a positive or doubtful reaction in 1 or more of the pools was individually tested with antigens within the pool. Titers to the individual antigens were obtained by diluting the sera 1:5 and placing 0.04, 0.02, 0.01, and 0.005 ml amounts on a slide. A drop of the desired antigen was added to each and the dilutions were mixed on a mechanical rotator (125 rpm for 4 min) and read on a Minnesota testing box which utilizes a dark background and an indirect light source. Reactions were graded as negative, +1, +2, +3, or +4. The highest dilution with a +1 reaction was regarded as the end point and the slide test titer was transposed to a microscopic test titer according to the method described by Galton *et al.*, (1958).

RESULTS

From 376 rodents trapped, 352 sera samples were obtained for leptospirosis testing with the ST and 326 kidney samples were obtained for culture. Table 1 lists the species represented and indicates the negative serologic and culture findings for the animals tested. Table 2 gives the domestic animal species, the location, and the number of positive animals, based on the ST. Positive goats and cattle were found in each locale sampled, while there were no positive sheep in the locations sampled. Table 3 lists the frequency of infecting serotypes. In animals with a titer to more than 1 serotype, the strongest titer was considered to be the infecting serotype. Multiple or dual infections and the possibility of paraspecific responses, where an animal exhibits a higher titer to an antigenically related serotype, cannot be discounted since

culturing was not accomplished in the domestic animal species. *Autumnalis* was the most frequently indicated serotype and accounted for 77.7% of the positive reactions in cattle and 83.4% of the positive reactions in goats. Fifteen of 36 (41.7%) of the cattle reacted to more than 1 serotype.

DISCUSSION

This study revealed the absence of any evidence of leptospira infection in rodents in the areas sampled. Although the ST is not as accurate as the microscopic agglutination test in assessing the amount of infection in a rodent population, it will definitely give presumptive data on the probable amount of infection in a given area (Sulzer *et al.*, 1968). When the ST test is augmented by kidney cultures, the data obtained should be a fairly accurate reflection of the amount of leptospirosis present in the populations sampled. The incidence of leptospirosis in rodents has varied greatly in different reports from different geographical areas (Tsai *et al.*, 1971 & 1973; McGuire and Meyers, 1957; Fresh *et al.*, 1968; Barsoum *et al.*, 1973), but the only information we found in the literature on rodent serology in Ethiopia, is a negative finding from 6 *Rattus* animals from Gambela in western Ethiopia (Moch *et al.*, 1975). Recent studies in Egypt (Barsoum *et al.*, 1973) revealed the presence of leptospirosis in *Mus musculus*, but all other rodent species including *Rattus*, *Arvicanthis*, and *Acomys* were negative. Earlier studies in Egypt (Brownlow and Dedeaux, 1964) revealed an 8.3% seropositivity and a 0.23% culture positivity on rodent species.

False negative culture results may be a result of faulty technique; the techniques used in this study, however, were verified by using known positive samples derived from stock cultures. It is also possible for an animal not to shed organisms but still have an antibody titer to the organisms (Sulzer *et al.*, 1968).

False negative serology may be the result of prozone phenomenon (Stoerner 1954), the sample being taken too early in the course of the disease (Alexander *et al.*, 1970), or the samples could be from carrier animals which have a very low or non-existent titer to leptospire which are found in their kidneys or urine (Alexander *et al.*, 1963; Birnbaum *et al.*, 1974). Brownlow (1964) also states that negative serology may be obtained when an animal has had a low grade infection of short duration. These causes for false negatives emphasized our need to combine serologic testing with culture attempts thus minimizing the chance of inaccurate reporting.

Previous studies by Moch and co-workers (1975) reported higher incidences of positive domestic animal serology in Ethiopia than were found in this study. They found a 71% incidence in cattle, 47% in goats, and 43% in sheep as compared to 26.3%, 16.7%, and 0%, respectively, in our present study. The differences may be due to the fact that we did not test for *butembo* and *tarassovi* serotypes which accounted for

71% of their positive reactions in those species. Both studies had a high percentage of bovine sera that reacted to more than 1 serotype (Moch, 48.8%; present study 41.7%).

Autumnalis was the predominant serotype in 77.7% (28 of 36) of the positive cattle and in 83.4% (5 of 6) of the positive goats. Although Moch found *autumnalis* in only a few sheep and not in other species, other studies in Africa have shown this serotype to be present in cattle and goats (Maronpot and Barsoum, 1972).

The differences in serotypes found by Moch may be due to the differences in the antigens used and possibly, to the fact that they were using the more sensitive microscopic agglutination test. There also could be climatic and geographic factors involved, since all the samples in our study were taken from the southwest quadrant of the country, while only 17 of the cattle, 2 of the sheep, and 1 of the goat samples, in Moch's study, were from this area. Further testing will be needed to answer the question.

SUMMARY

Wild rodents were trapped in 5 provinces of southwest Ethiopia. Serologic testing for leptospirosis, using the macroscopic slide agglutination test, failed to reveal any sera positive to the 18 antigens represented in the test. Negative kidney cultures reinforced the negative serologic findings. Sera collected from cattle, goats, and sheep in 3 of the areas, revealed leptospira titers of 1:16, or greater, in 26.3% of the cattle, 16.7% of the goats, and in none of the sheep. The most frequently indicated serotype was *autumnalis*. Fifteen of 36 (41.7%) of the positive cattle had antibodies to more than 1 serotype.

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LEGENDS

Fig. 1. Locations in Ethiopia where animals were trapped and samples obtained.

TABLES

- Table 1: Results of rodent kidney cultures and macroscopic slide test screening with pooled antigens.
- Table 2: Domestic animal serologic results.
- Table 3: Domestic animal infecting serotype frequencies.

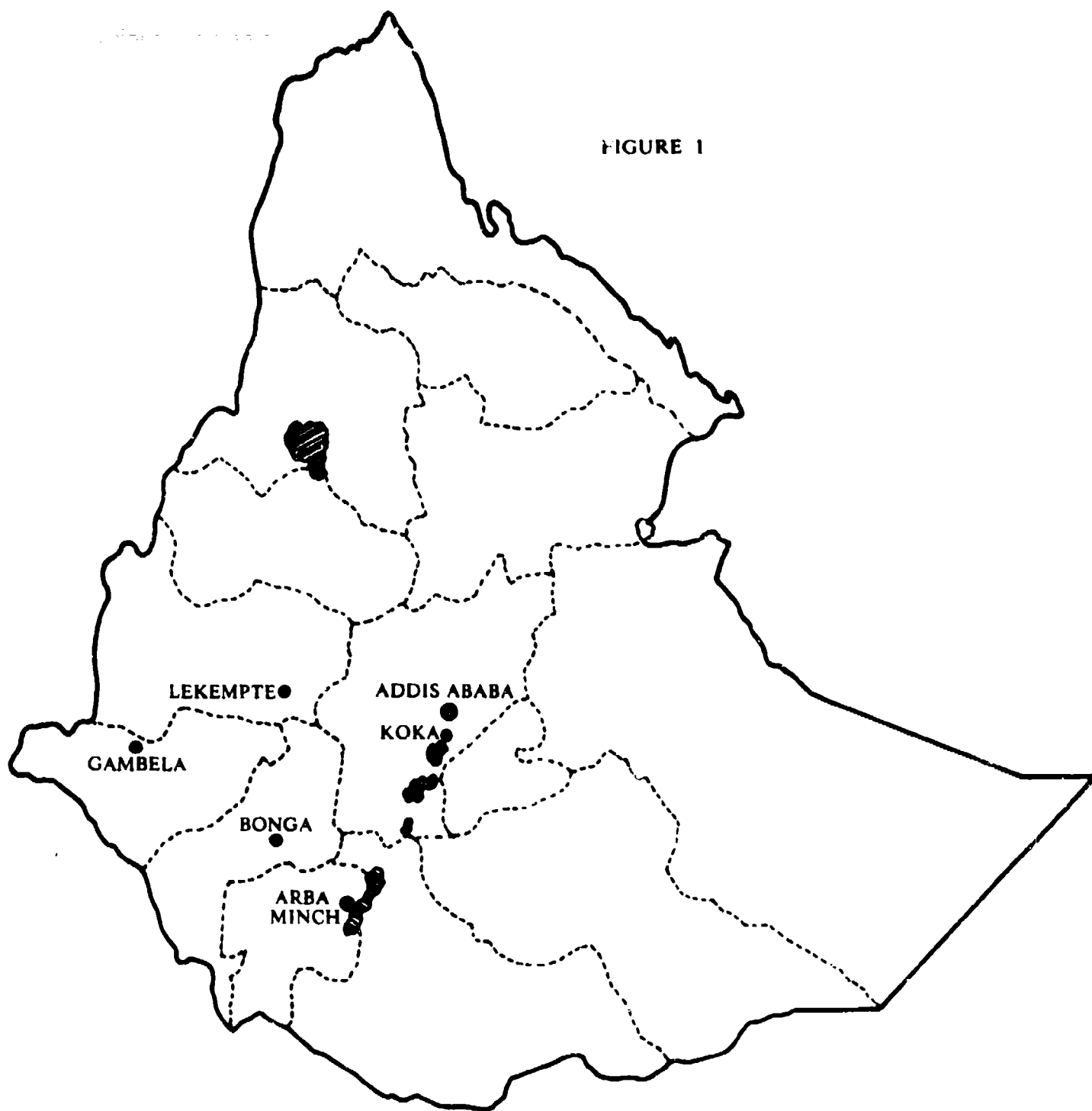


Fig. 1. Locations in Ethiopia where animals were trapped and samples obtained

TABLE 1

Results of rodent kidney cultures and macroscopic slide test screening with pooled antigens.

Species	Macroscopic slide Test			Culture	
	Total	Positive	Negative	Positive	Negative
<i>Arvicanthis dembeensis</i>	114	-	113	-	92
<i>Rattus rattus</i>	194	-	179	-	175
<i>Mastomys natalensis</i>	56	-	51	-	47
<i>Acomys cahirinus</i>	8	-	5	-	8
<i>Leuphoromys flavopunctatus</i>	3	-	3	-	3
<i>Praomys natalensis</i>	1	-	1	-	1
TOTAL	276	-	352	-	326

TABLE 2

Domestic animal serologic results.

Species	Location	Number Tested	Number Positive*	Percent Positive
Bovine:	Gambela	23	4	17.39
	Lekempte	55	15	27.27
	Arba Minch	59	17	28.81
	TOTAL	137	36	26.27
Ovine:	Gambela	10	0	0
	Lekempte	21	0	0
	TOTAL	31	0	0
Caprine:	Gambela	8	1	12.50
	Lekempte	10	3	30.00
	Arba Minch	18	2	11.11
	TOTAL	36	6	16.66

* A 1:16 or greater slide test titer was considered positive.

TABLE 3

Domestic animal infecting serotype frequencies.

Bovine serotypes		Caprine serotypes	
<i>autumnalis</i>	28	<i>autumnalis</i>	5
<i>sejroe</i>	3	<i>icterohemorragiae</i>	1
<i>pomona</i>	2		
<i>wolffii</i>	2		
<i>mini,georgia</i>	1		